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Bird Repellents: Interaction of Chemical Agents in Mixtures

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CLARK, L. *Bird Repellents: Interaction of chemical agents in mixture.* PHYSIOL BEHAV **64** (5) 689–695, 1998.—Numerous studies characterize the concentration–behavioral response for odorants, tastants, and irritants. However, to achieve ecological validity, interaction of agents in mixtures must be considered. Equiresponse and equimolar molar models of interactions have been proposed, and methods for testing whether agents in mixture interact independently have been evaluated. Yet these averaging models cannot a priori predict whether agents will interact antagonistically, independently, or synergistically. I studied the bird repellent properties of several structurally similar and well-described trigeminally mediated avian irritants, singly and in mixture. Compounds within a chemical class, in which the electron withdrawing groups were similar, interacted independently to produce their repellent effects, e.g., 2-amino methyl benzoate v methyl-2-methoxy benzoate, and *o*-aminoacetophenone v 2-methoxy acetophenone. The response to mixtures drawn from compounds of dissimilar chemical class, e.g., 2-amino methyl benzoate v *o*-aminoacetophenone, interacted antagonistically at concentrations below 10 mM, suggesting meditation by a different mechanism within the trigeminally mediated sensory modality. At 10 mM and near saturation of the solutions, there was no evidence of interaction between agents, suggesting responses became saturated. These observations underscore our previous findings for the importance of the molecular properties of the carbonyl group for aromatic bird repellents and suggests the possible existence of multiple receptor mechanisms for avian trigeminal repellents. These data also underscore the importance of attending to interactions of agents in mixtures when designing repellents as tools for the management of wildlife and resolution of conflicts between humans and wildlife. © 1998 Elsevier Science Inc.

Bird Mixtures Irritation Repellent Trigeminal

THERE is a historically strong emphasis in the chemical senses for describing the relationship between concentration and intensity of response for single agents. This emphasis is due to the straightforward design of such studies and the simplicity by which the concentration–response relationship can be modeled. Yet, it also is recognized that to achieve ecological validity, interaction of agents in mixture must be considered (24). For various reasons, single agent concentration–response relationships are useful, particularly because it is believed that the behavioral response to a mixture can be predicted from the concentration–response relationships of its individual constituents. The validity of this approach hinges upon the assumption that components of a mixture share a common receptor mechanism and that there is a direct correspondence between mechanism and the physiological or behavioral assay (14).

For single agents that are perceptually similar, averaging models have proven useful as predictors of behavioral activity for mixtures (15,25,30). In averaging models, the magnitude of the response to a mixture lies between the responses to the unmixed equiconcentration components. Moreover, electrophysiological studies indicate that so long as the single agents are cross-adaptive, the averaging models work equally well for gustatory and olfactory stimuli (19). Thus, the principles underlying the mechanism of interaction appear to be independent of sensory modality. When agents in mixture are mutually noninterfering in their effect, as predicted by averaging models, they are considered to be additive or independent. However, many behavioral studies show that the perceived intensity of mixtures is less than the sum of its parts. The diminution of effect is presumed to result from complete or partial antagonism of agents in mixture. Equivalent terms appearing in the literature are suppression, inhibition, or hypoadditity (26,27). Other studies show that a mixture frequently is perceived as more intense than the sum of its parts. Equivalent terms are synergy, enhancement, or hyperadditivity (3).

Studies indicative of nonadditive interactions of agents within mixtures indicate that components with little cross-adaptivity typically yield enhanced responses, presumably because of the simultaneous activation of independent receptor sites by the different components of the mixture (19), or yield suppressed responses because of partial or complete antagonism by the components of the mixture at the common receptor sites (13). Furthermore, although there is a tendency to consider the interactions of agents in mixture to be constant, this often is not the case. The interaction of agents in mixture often yield an effect on the behavioral response

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that varies as function of concentration of the mixture and the ratio of the agents in the mixture (11). Unfortunately, in the absence of detailed binding or physiological studies, there is no basis for the a priori prediction of the behavioral response of an animal to a mixture based upon the shape of the individual components' concentration–response relationship. This is lamentable for a variety of practical considerations.

The investigation of nonlethal chemical repellents as tools for wildlife conservation and management has attracted increasing attention as a means to resolve conflicts between wildlife and humans (23). This field is relatively undeveloped, and most of the studies have focused on the avoidance response of wildlife to single agents in an effort to empirically catalog the concentration-response relationships of candidate repellents to target lead compounds for future development (5). However, there may be circumstances in which combinations of repellents in a single formulation may be indicated. For example, target levels of repellency for single agents may be achieved, but only with acute or chronic risks of toxicity. In this case, a combination of repellents may be desirable if the toxicity of the combined partial doses of the two or more repellents in mixture is less than with full doses of any single component of the mixture.

Given the dearth of information on the interaction of repellents in mixture (18,22), I set out to: 1) characterize the concentration–response relationship of well described, trigeminally mediated bird irritants (4,5); 2) empirically determine the interaction effect of binary mixtures of these irritants; and 3) determine whether the type of interaction was related to the chemical and structural similarity of components in binary mixture and, by implication, a function of similar mediating mechanism for the repellents under consideration.

MATERIALS AND METHODS

Subjects

Adult European starlings *(Sturnus vulgaris)* were captured at the United States Department of Agriculture/Denver Wildlife Research Center field station at Sandusky, OH and transported to the Monell Chemical Senses Center. Starlings were individually caged (61 × 36 × 41 cm) under a 12:12 light:dark cycle for a 2-week adaptation period and given free access to Purina Flight Bird Conditioner (Purina Mills, St. Louis, MO, USA), water, and oyster shell grit. Starlings were chosen as test animals because previous experiments showed them to be good models of avian chemosensitivity (7).

Test Protocol

After a 2-week adaptation period, 36 experimentally naive starlings were randomly assigned to one of six groups, and water consumption was monitored every 2 h over a 6-h period over the course of 3 days to ensure that consumption was within the normal range empirically established for starlings under test conditions in our laboratory. Birds deviating more than ±2 SD units from a seasonally adjusted mean are excluded from experiments (such birds are often hyperactive, or alternatively, sick); no birds needed to be excluded from these experiments. Similarity of mean consumption among groups was verified using a two-way repeated measures (on days) ANOVA and was a precondition for additional testing.

Groups were randomly assigned to receive one of six concentrations of the test solution. Maximum concentrations tested were bounded by the water solubility limits of the test compounds. The other concentrations tested were determined by geometric-step serial dilutions. Based on previous studies for these test compounds, this procedure insured the incorporation of concentrations yielding minimum and maximum asymptotic responses (4). Fluid intake was monitored every 2 h (to check for spillage) for a total of 6 h to verify that avoidance was attributable to primary, and not postingestional, effects (5,29). At the end of the experiment, birds were returned to group housing, and the process was repeated for the next test compound.

Stimuli

A total of nine separate drinking trials were carried out with five single-component solutions, and four 1:1 equimolar binary-component solutions. Test compounds were all potent bird repellents (5). Included were the 2-amino and 2-methoxy moieties of acetophenone and methyl benzoate, as well as an outlier terpenoid structure. The test compounds were: *o*-aminoacetophenone (OAP, CAS # 551–93-9), methyl anthranilate (MA, CAS # 134–20-3), methyl-2-methoxybenzoate (M2MOB, CAS # 606–45-1), 2-methoxyacetophenone (2MAP, CAS # 4079–52-1), and D-pulegone (PUL, CAS 89–82-7).

Analysis

The terms for performance of repellents are adopted directly from the pharmaceutical sciences (16). Efficacy (E) is defined as the ability of the repellent to reduce fluid intake relative to the control ($p < 0.05$). Maximum efficacy (E_{max}) is the maximum suppressive effect of the repellent. An additional index of efficacy is used for repellents (7) that incorporates the evaluation whether E_{max} is statistically indistinguishable from zero consumption ($^{0}E_{max}$). Measures of potency incorporate information about the concentration that is necessary to achieve a specified definition of efficacy (P_E, P_{Emax}, and P^{0}_{Emax}, respectively).

Multiple models for the concentration–response relationship were considered. The model that minimized the mean SE term using a Hooke and Jeeves, quasi-Newton iterative optimization technique (STATISTICA, '93) based upon concentration group averages was:

$$R = a/(1 + (x/c)^{b}) \quad [1]$$

where R is the relative intake of a repellent solution as compared to a fresh water control, a is the asymptotic maximum relative intake, b is the slope, c is the inflection, and x is the test concentration. Slope is a useful index of sensitivity of birds to changes in the effects of a repellent. Inflection is a useful standard comparative index of potency of repellents providing that the curves are parallel, i.e., slopes are equal.

Concentration–response relationships with similar slopes are presumed to be mediated by similar receptor mechanisms (16). Shifts in concentrations for inflection or substrate saturation (minimum asymptote) may merely reflect differences for the agent's affinity for the receptor mechanism. Comparisons for similarity of slopes (overlap of the 95% confidence intervals) were used to test the assumption that repellency acts through the same effector system via similar mechanisms. While similarity of slopes does not prove similarity of mode of action, it is consistent with such an interpretation. Different slopes for the concentration–response curves would strongly suggest a different effector and/or mechanism mediating the avoidance response (10).

A variety of models have been used to describe the interaction of components in mixture (24,30). Averaging models have the most utility and robustness, providing that an appropriate model for the concentration–response relationship is used. Such models are derivative of equimolar models (EMM) or isobole approaches, where the index of interaction is based upon averaging the con-

TABLE 1

SUMMARY OF REGRESSION MODEL PARAMETERS FOR THE DOSE–RESPONSE RELATIONSHIPS

	R^2	Max[a]	Slope	Inflection	P_E	E_{max}
Single Component						
PUL	96.8	0.92 (.05)	−3.03 (1.14)	5.96 (0.78)	5.76	0.00
MA	98.7	1.09 (.07)	−1.24 (0.03)	5.29 (1.50)	1.72	0.12
M2MOB	98.3	1.07 (.08)	−1.20 (0.25)	3.91 (0.90)	2.63	0.00
OAP	98.8	1.06 (.07)	−0.85 (0.18)	1.93 (0.65)	0.25	0.16
2MAP	98.9	1.01 (.05)	−1.77 (0.34)	3.75 (0.43)	5.25	0.11
Mixtures[b]						
MA-PUL	95.9	1.00 (.04)	−2.60 (0.67)	10.22 (1.16)	10.50	0.12
MA-M2MOB	98.6	1.06 (.19)	−1.42 (0.64)	7.28 (0.88)	1.31	0.18
MA-OAP	99.0	0.99 (.04)	−2.88 (0.59)	10.30 (0.74)	10.50	0.19
OAP-2MAP	98.7	1.11 (.15)	−0.95 (0.30)	4.09 (1.83)	7.00	0.24

R^2, percentage of variance explained by the four-parameter nonlinear regression model.

[a] The maximum asymptotic relative fluid intake.

[b] Binary test solutions were 1:1 ratios of equimolar component solutions.

centrations necessary to produce the same effect. This approach presumes to compensate for differential access to receptors due to physicochemical characteristics of stimulus molecules. I chose to use an averaging model as the basis for determining the type of interaction between components in mixture with respect to the avoidance response at equal concentration because there is no certainty whether the differential concentration–response is due to accessibility or with differences in receptor mechanism. Furthermore, because my interest is in the overall behavioral response of potentially practical repellents, the most useful metric in evaluating formulations is the integration of components of access and mechanism.

The interaction of individual agents and their mixtures is described as follows. $R_{[A],i}$ and $R_{[B],i}$ are the responses to agents A and B at concentration i, respectively. $R_{[AB],i}$ is the response to the mixture of agents A and B at concentration, i. For the purposes of this study, the mixtures A:B are all 1:1. The actual composition ratio is not of importance so long as the responses are monotonic (2,31). The theoretical null condition of independence of agents in mixture for concentration, i, is defined as:

$$R^o_{[AB],i} = (R_{[A],i} + R_{[B],i})/2 \quad [2]$$

and the interaction index for concentration, i, is defined as:

$$I = R^o_{[AB],i} - R_{[AB],i} \quad [3]$$

where no interaction occurs between the agents when $I = 0$. When $I < 0$, the interacting agents are antagonistic. When $I > 0$ the interacting agents are synergistic. This equimolar approach is analogous to the isobole approach of equal response as an empiric description of the interactions of agents in mixture on the concentration-response relationship (2,31), and does not imply any specific mechanism for coding the stimulus.

Given the statistical uncertainty of estimating the fitted concentration-response curve parameters, and hence the values of R and the derived index, I, I used a conservative evaluation of whether I differed from the null condition, $I = 0$. Agents in mixture were presumed to have no interaction if the 95% confidence limits of I_i encompassed zero.

RESULTS

Baseline Water Intake

No group differences were detected during any of the nine tests of single agents or binary solutions ($p > 0.90$, respectively). Thus, presentation with each of the bird repellents or mixtures proceeded as planned. A retrospective two-way repeated measures analysis of variance of group and time effects (where each test solution was tested at different times) showed that the pattern of baseline water consumption among groups did not differ across time (i.e., the interaction effect had a $p = 1.0$). Baseline water consumption for groups collapsed across time was similar ($p = 0.965$). However, baseline water intake collapsed across groups differed among test dates ($F = 5.895$, df = 7,210, $p < 0.001$). A post hoc test showed that water consumption was lower during the week that M2MOB and M2MOB/MA were tested. Unidentified extraneous factors may have caused this slight temporary shift in baseline water consumption. Because of this difference among test dates, the intake of chemically treated test solutions was standardized to the fluid intake of a control group of birds presented with tap water.

Concentration–Response Relationships

The three parameter logistic model accounted for between 95.99 and 98.97% of the variance of the concentration–response relationship for all test compounds and their mixtures (Table 1). In addition, the pattern of fluid intake of test solutions was consistent with a pattern expected for a primary repellent (5), i.e., for a given concentration, the bihourly intake was constant. This pattern indicates that no learned avoidance occurred. Rather, avoidance was sensorially mediated and primarily driven by concentration effects (Fig. 1). Thus, neither the interaction term (time–concentration) nor the main effect of time achieved $p < 0.1$ for any of the compounds or mixtures. Each of the test solutions resulted in a concentration-dependent repellent effect ($E = H_o$: $R = 1$; df = 5, 30): MA ($F = 13.822$, $p < 0.001$); M2MOB ($F = 19.790$, $p < 0.001$); OAP ($F = 15.696$, $p < 0.001$); PUL ($F = 3.184$, $p < 0.05$); MA-M2MOB ($F = 44.797$, $p < 0.001$); MA-OAP ($F = 9.621$, $p < 0.001$); MA-PUL ($F = 5.192$, $p < 0.002$); OAP-2MAP ($F = 5.269$, $p < 0.001$). In all cases, the shapes of the concentration–response curves for single component solutions (Table 1) compared favorably to parameter values calculated previously for these compounds (cf. Refs. 7–9).

The slopes of the concentration–response curves for each of the test solutions were similar, i.e., the slope parameter values of single agents and their mixture had overlapping 95% confidence intervals, with the exception that the slope for the concentration–response curve of the MA/OAP mixture differed from the slopes for the curves for either of its components, which themselves were

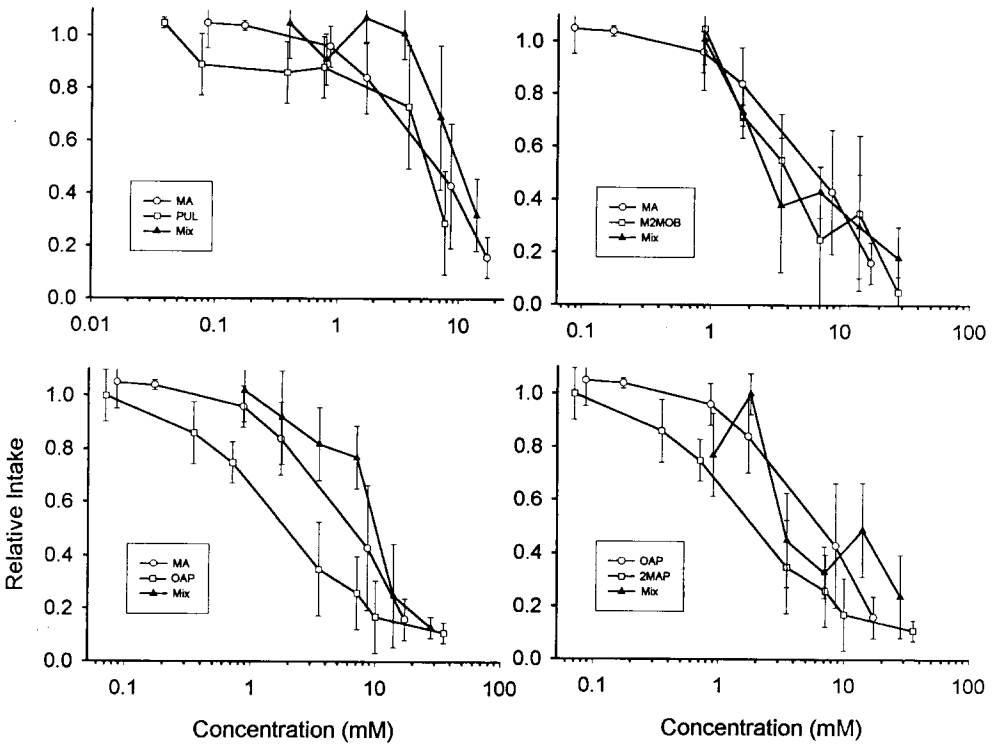

FIG. 1. Average 6-h fluid intake by starlings as a function of concentration for single agents or binary mixtures. $n = 6$ for each concentration tested. Vertical capped bars depict ± 95% confidence limits. Refer to Methods for chemical codes.

similar (Table 1). The similarity for the slopes of single agent solutions for considered pairs suggested similar mechanisms governing rate dynamics of the concentration–response relationship (16) and allowed a direct comparison of potency using the inflection point.

The potencies of the equimolar single agent solutions composed of the aromatic (MA) and terpenoid (PUL) were similar. However, the potency of a 1:1 equimolar mixture of the two agents was lower than was observed for PUL, but not for MA (Fig. 2A). The reason the rightward shift of the concentration-response curve of the mixture was different from that of PUL but not MA was due to the smaller error associated with the inflection for the former (Table 1). The potencies of the aromatic benzoate structures, MA, M2MOB, and their mixture were similar (Fig. 3A, Table 1), as were the potencies of the aromatic acetophenone structures, OAP, 2MAP and a 1:1 mixture of the two (Fig. 4A). OAP was more potent than equimolar solutions of MA and the MA/OAP mixture. There was no difference between the potencies of equimolar solutions of MA and the MA/OAP mixture (Fig. 5A).

The interaction of structurally similar chemicals in mixture, i.e., the two benzoates (Fig. 3B) and two acetophenones (Fig. 4B), was independent over the entire concentration range tested, as judged by the inclusion of the null condition of $I = 0$ within the 95% confidence bands of the mixtures' interaction term. Moreover, the interaction indices for these mixtures deviated less than 6% of full scale from the null condition of no interaction across the range of concentrations tested.

In contrast, the interaction for dissimilar chemicals in mixture, i.e., MA/PUL (Fig. 2B) and MA/OAP (Fig. 5B), was antagonistic for all but the highest concentrations tested. At the solubility limit for the MA/PUL mixture the interaction tended toward independence, i.e., the 95% confidence interval for the mixture's interaction term encompassed the null condition of $I = 0$. Even with a large degree of uncertainty for the value of the interaction index as indicated by the broad 95% confidence limits, the interaction of MA and PUL in mixture at concentrations below 10 mM appeared to be antagonistic (Fig. 2B). At 10 mM, a concentration range that is characterized by a significant suppression for fluid consumption relative to controls (60%), the deviation from the null condition of no interaction is 25% of full scale. That is to say, birds consumed 25% more fluid of the MA-PUL mixture, i.e., it was less repellent, than would be expected from simply averaging the single agent concentration-specific responses of the individual components of the mixture.

Similarly, at the solubility limit for the MA-OAP mixture, the behavioral response by birds indicated no interaction between agents. At concentrations less than 15 mM, the agents in mixture were antagonistic (Fig. 5B). At 10 mM fluid consumption of the mixture was suppressed by 80% relative to controls (Fig. 5A), but this was 10% more than would be predicted on the basis of averaging the concentration–response curves of the mixture's individual components. At 5 mM, the mixture suppressed fluid intake by 50% relative to the plain water (Fig. 5A), but this level of consumption was 20% more than would be expected on the basis of averaging the concentration-response curves of the mixture's individual components.

DISCUSSION

For perceptually similar compounds, averaging models have proven to be useful predictors of behavioral responsiveness to mixtures (12,30). Detailed physiological studies of sensory neurons show that stimuli that are cross-adaptive tend to operate additively (i.e., independently) in mixture, regardless of the sen-

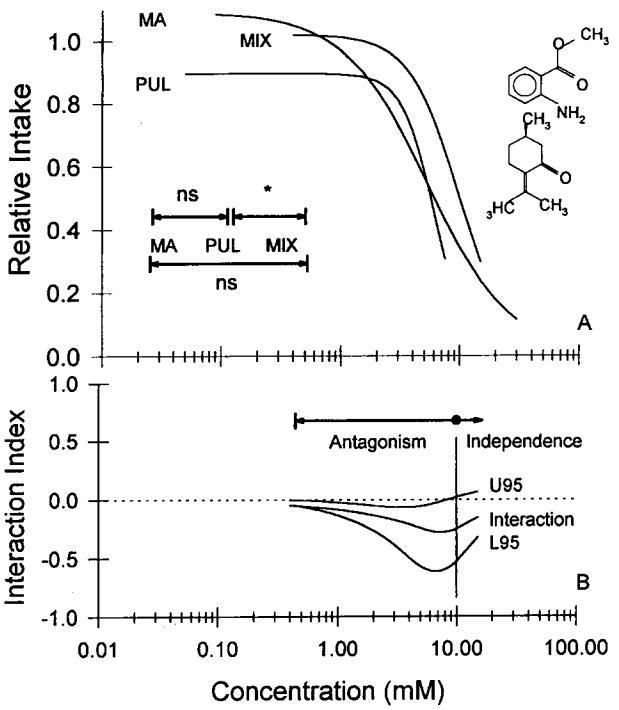

FIG. 2. (A) Fitted curve for the concentration–response relationship for methyl anthranilate, pulegone, and the 1:1 equimolar mixture of the two. Structures for the test agents are indicated. The slopes of the curves were similar. Comparison of potencies (i.e., positional concentration shifts) for the curves are indicated in the inset (*$p < 0.05$; not significant, $p > 0.05$). (B) the interaction index for behavioral responding to agents in mixture (center curve) along with the upper (U95) and lower (L95) confidence limits as a function of mixture concentration. The horizontal dotted line depicts the null condition of zero interaction (independence) between components in mixture. The vertical line separates the concentration ranges indicative of antagonistic and independent interactions of components in mixture.

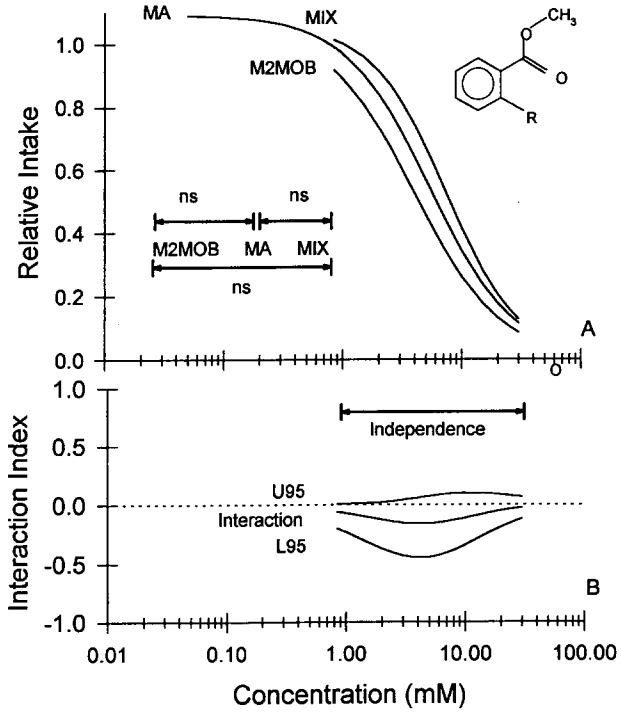

FIG. 3. (A) Fitted curve for the concentration-response relationship for methyl anthranilate (MA), methyl-2-methoxybenzoate (M2MOB), and the 1:1 equimolar mixture of the two. Structures for the test agents are indicated (R = NH_2 for MA and OCH_3 for M2MOB). The slopes of the curves were similar. Comparison of potencies (i.e., positional concentration shifts) for the curves are indicated in the inset (not significant, $p > 0.05$). (B) the interaction index for behavioral responding to agents in mixture (center curve) along with the upper (U95) and lower (L95) confidence limits as a function of mixture concentration. The horizontal dotted line depicts the null condition of zero interaction (independence) between components in mixture. The agents in mixture were interpreted as effecting a response independent of one another.

sory modality (19). However, in the absence of empirically intensive studies of cross adaptation, there is little predictive power for the nature of interaction of components in mixture based upon bioassays for responsiveness to single agent stimuli. The isobole approach used here for describing the interaction of agents in mixture uses concentrations, effects, and empiric concentration-effects relationships, and is independent of the mechanism of interaction (31). This approach is useful because it can be applied to systems for which mechanistic information driving the concentration-response is not available.

Use of the isobole approach has been criticized by investigators in the chemical senses (20,21) because the interaction term often varies as a function of concentration. It would appear that such a criticism could be levied against this study because I showed that the nature of interaction for MA-OAP and MA-PUL mixtures varied as a function of concentration. Mathematical arguments about the instability of the isobole interaction notwithstanding, there is ample experimental evidence to indicate synergism in one concentration range and antagonism in another (i.e., the "Fechner paradox"; Reference 17), not only for the chemical senses (3) but more broadly in the pharmaceutical sciences (16). Thus, it is more likely that the underlying assumption of constant interaction across concentrations is not valid, hence the models constructed around this approach may not adequately describe the nature of the interaction (31).

If averaging models and constancy of the interaction term are not adequate to explain the data, then what recourse do we have for generating a predictive model? Molecular modeling techniques, which have proven useful in predicting levels of activity and qualitative perception of homogeneous sets of stimuli, may be of utility for sensory studies of mixtures. The usefulness of a molecular modeling approach should not be surprising because, ultimately, the electronic, topological, and physicochemical features of molecules are the basis for interaction with receptor mechanisms (28). Yet surprisingly, the molecular modeling techniques have not been used extensively to make predictions how single agents may interact in mixture.

Although pharmacological studies suggest that the slopes of individual concentration–response curves are useful indicators for determining the similarity of receptor mechanisms, this study showed that the placement and character of the substituent on a parent aromatic structure is more important to the nature of interaction of aromatic components within a mixture. Compounds defined by substituents within the electron withdrawing group (EWG), e.g., anthranilates (R = OCH_3), acetophenones (R = CH_3; Fig. 3, and 4), interacted independently, suggesting a single receptor mechanism mediated the behavioral response within each of their respective chemical classes. This is consistent with the averaging models of Beidler (1) and the subsequent derivations. Also

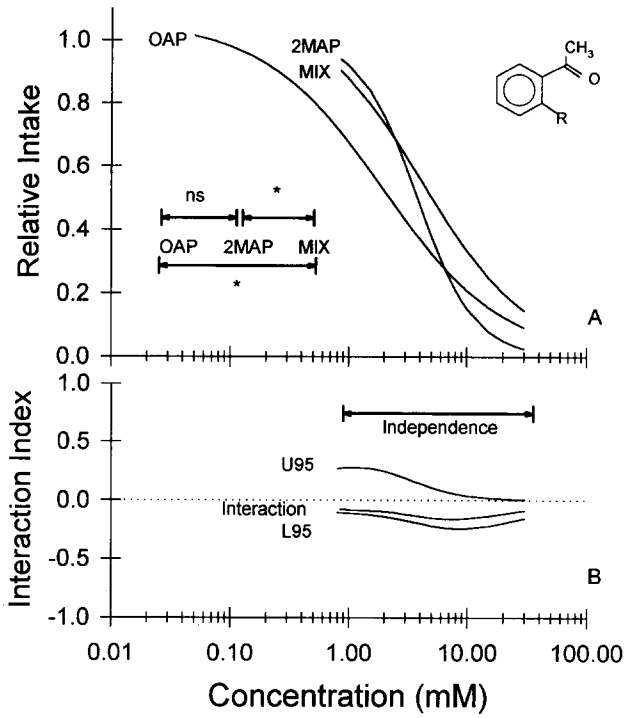

FIG. 4. (A) Fitted curve for the concentration–response relationship for *o*-aminoacetophenone (OAP), 2-methoxyacetophenone (2MAP), and the 1:1 equimolar mixture of the two. Structures for the test agents are indicated (R = CH_3 for OAP and OCH_3 for 2MAP). The slopes of the curves were similar. Comparison of potencies (i.e., positional concentration shifts) for the curves are indicated in the inset (not significant, $p > 0.05$). (B) the interaction index for behavioral responding to agents in mixture (center curve) along with the upper (U95) and lower (L95) confidence limits as a function of mixture concentration. The horizontal dotted line depicts the null condition of zero interaction (independence) between components in mixture. The agents in mixture were interpreted as effecting a response independent of one another.

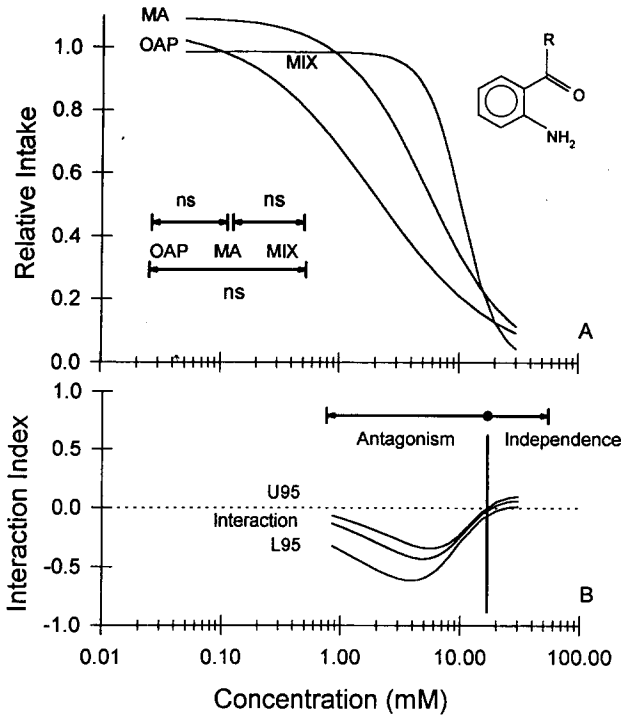

FIG. 5. (A) Fitted curve for the concentration–response relationship for methyl anthranilate (MA), *o*-aminoacetophenone (OAP), and the 1:1 equimolar mixture of the two. Structures for the test agents are indicated (R = OCH_3 for MA and CH_3 for OAP). The slopes of the curves were similar. Comparison of potencies (i.e., positional concentration shifts) for the curves are indicated in the inset (*$p < 0.05$; not significant, $p > 0.05$). (B), the interaction index for behavioral responding to agents in mixture (center curve) along with the upper (U95) and lower (L95) confidence limits as a function of mixture concentration. The horizontal dotted line depicts the null condition of zero interaction (independence) between components in mixture. The vertical line separates the concentration ranges indicative of antagonistic and independent interactions of components in mixture.

consistent with the averaging models and the pharmacological model is the notion that similar slopes for the single agents within each of these classes of compounds (OAP versus MAP and MA versus M2MOB) will result in independent interaction if these agents occur in mixture. However, when the EWGs differed, e.g., OAP versus MA, the interaction of these components in mixture was antagonistic for all but the very highest concentrations tested (Fig. 2). Thus, the contribution of the electron donating group seemed to be more important in determining the level of efficacy within a chemical class (8). Similarly, the structurally unrelated compounds, MA and PUL, also were antagonistic for concentrations below 10 mM. The lack of interaction for concentrations near the solubility limit for components in these two mixtures suggests that the response may become saturated. Slope was not correlated to the type of interaction of the components in mixture.

These observations underscore the interpretation that, based upon the concentration–behavioral response, the similarity of slopes in this instance may not be used to infer similar mechanisms mediating shifts in the concentration-specific response to mixtures. However, consideration of electronic features of the stimuli appear promising for predicting the behavioral response. Furthermore, the structure–activity approach may prove useful for identifying important features of ligands and, by implication, be import for postulating structures for the receptor complex. Most importantly, these data show that while mediating sensory mechanisms may be similar, i.e., trigeminally mediated (4,6), the mechanism within the sensory system may differ as a function of stimulus class. This possibility warrants further consideration.

Practically, these data suggest that molecular modeling of molecules may have use in predicting whether or not the components in mixtures will conform to the null hypothesis of independence based on averaging models over an approach that uses similarity of slopes of the single agent dose–response relationships. Moreover, these findings suggest that caution should be exercised when considering mixtures of active ingredients in the development of repellent formulations. While synergistic interactions may be desired because they would lower concentrations of active ingredients to produce a given level of effect, there remains a distinct possibility that the opposite outcome may result when active agents interact antagonistically. The data presented in this study indicate that more thorough studies elucidating the concentration-specific interactions of agents in mixture are needed prior to making recommendations on formulation of repellents. The field of wildlife management is in its infancy in understanding why individual agents or mixtures of repellents succeed or fail. Rational design for formulations is becoming increasingly critical as resources for laboratory and field evaluation studies dwindle and the

pressure to find effective nonlethal repellent formulations to resolve wildlife–human conflicts increases. Careful consideration focusing on the composition of repellent formulations prior to field studies would greatly reduce research and development costs and improve the likelihood successful development of such formulations.

SUMMARY

Birds avoided mixtures of irritating stimuli according to predictions of sensory averaging models only when the stimuli were of the same chemical class. When irritating stimuli in mixture were of different chemical classes (MA + OAP), the concentration-dependent avoidance response was less than that predicted from averaging models at concentrations less than 10 mM, indicating that the agents in mixture were antagonistic. At higher concentrations, there was no interaction between agents, suggesting a saturated behavioral response. These data suggest that the nature of the interaction is complex and varies owing to attributes of the chemical species combined in mixture and their concentrations. However, at concentrations below the behavioral saturation response, chemical structural data may provide clues about the nature of interaction of agents in mixture. This latter point warrants further study. Because our current knowledge of how agents might interact is limited careful laboratory consideration about the nature of interactions of agents in mixture is warranted prior to proceeding onto more expensive field evaluations.

ACKNOWLEDGEMENTS

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